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(54) Title: **RAPID NUCLEIC ACID SEPARATION, ISOLATION AND PURIFICATION METHODS**

(57) Abstract: Provided are rapid and simple methods for isolation and purification of nucleic acid from crude samples.



WO 01/62976 A1

Rapid Nucleic Acid Separation, Isolation and Purification Methods

BACKGROUND OF THE INVENTION

I. Field of the Invention

The present invention relates to the isolation and purification of nucleic acids.

II. Background

Methods for nucleic acid isolation and purification have existed and been known for years. For example, ionic exchange resins have been used for purification of nucleic acid since 1950's (W.E. Cohn, Ann. N.Y. Acad. Sci., 57, p204, 1953). Nucleic acids, proteins and other impurities are attached onto solid support through anion exchange. Through washings, proteins and unwanted nucleic acids are removed. Nucleic acid is eluted in high salt (i.e. 7M urea or 1.2 M NaCl) (Henco, et al., US patent 5,057,426) and, then, further purified through ethanol precipitation. Gebeyehu, *et al.* eluted nucleic acid with 0.5 N NaOH (US patent 4,921,805) which denatures nucleic acid.

Ultracentrifugation with sucrose or cesium chloride density gradients is another method of separation of nucleic acid. (Alberts, *et al.*, Molecular biology of the Cell, p162, third edition, Garland Publishing, 1994) The nucleic acids are separated from other macromolecules according to their sedimentation coefficient. The sample is centrifuged at very high speed for 20 hours or longer.

Phenol or chloroform/phenol extraction and ethanol or isopropanol precipitations of nucleic acids are common lab procedures. (Short Protocols in Molecular Biology, p55, John Wiley & Sons, 1989). Phenol or chloroform/phenol extractions can result in contact with these toxic organic solvents and are time-consuming procedures. Alcohol precipitation of nucleic acid from solution involve the addition of salt (e.g. 0.1 volumes of 2.5M sodium acetate (pH 5.2)) to a solution containing nucleic acids followed by addition of an alcohol (e.g. 2.5 volumes of ethanol). The nucleic acids then precipitate. The precipitated nucleic acid molecules aggregate (usually at low temperatures; e.g. 5 minutes on dry ice or 30 minutes at 4°C) and are recovered by centrifugation for 30 minutes (usually at low temperature). This is a time-consuming process, and it is more useful to concentrate prepurified nucleic acids rather than a true purification method.

More recently, affinity purification procedures have been used in nucleic acid field. Nucleic acids and impurities are bound to solid support materials such as glass and diatomous materials through affinity binding in presence of a chaotropic agent (Padhye, et al, U.S. Patent 5,808,041; Little, US patent 5,075,430; Boom, et al, US patent 5,234,809) or mixture of chaotropic agent and an organic solvent (Wiggins, US patent 5,637,687; Chomczynski, US patent 5,945,515). However, this method has difficulty in ever tight bonding and difficulty in binding small molecules and releasing long nucleic acid. Thomas, *et al.*, (US patent 5,175,271) showed a method of DNA extraction with silica gel and amyl alcohol. Here amyl alcohol plays same role as phenol and chloroform.

Reeve (US patent: 5,665,554 and 5,681,946) discloses that magnetic beads can precipitate DNA or protein. These DNA or proteins are precipitated on the surface of magnetic beads. The magnetic beads are collected by applying magnetic field.

Woodard (US patent 5,405,951 and EP 0512767) teaches addition of alcohol to DNA solution. In presence of celite diatoms or silicas, the DNA will bind to celite diatom or silica. After drying, DNA is eluted with heating at 60°C. The binding and eluting steps need to repeat at least once. Therefore, the method is cumbersome, slow, and the recover is limited.

Mullis (US patent 5,187,083 and 5,234,824) reveals that certain membranes trap high molecular weight DNA. DNA is released in a buffer containing divalent ions such as calcium ions at elevated temperature.

Gel filtration is widely used in purification of nucleic acids. (J. Brobyt and B. White, Biochemical Techniques, page 88-95, Brooks/Cole publishing, 1987) It requires either natural gravity or centrifugation. The method of natural gravity is very time consuming, while centrifugation has limited capacity that is not suitable to high throughput purification. Furthermore, the purified samples are usually diluted after purification.

With rapid development of genome sequencing and diagnostic fields, with an increasing use of nucleic acid, speed of nucleic acid sample preparation becomes an important factor. Therefore, there is a great need to develop a nucleic acid purification, isolation and purification method which is quick, effective, and can be carried out at ambient temperature.

SUMMARY OF THE INVENTION

It is an object of this invention to provide methods for the convenient isolation and purification of nucleic acids from an aqueous sample.

It is a further object of the invention to provide a kit useful for isolation and purification of nucleic acids from an aqueous sample.

In accomplishing those objectives, there is provided a method of purification of a nucleic acid from a solution comprising

the mixing an aqueous sample solution comprising a nucleic acid, an organic solvent miscible in water, and a solid surface selected from the group consisting of an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20°C and an organic solid surface having a surface tension of 30-70 dyn/cm at 20°C;

collecting the solid surface on which the nucleic acid is precipitated; and
eluting the nucleic acid from the solid surface at ambient temperature.

In accordance with a preferred embodiment, the solid surface is an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20°C. In accordance with another preferred embodiment, the solid surface is an organic solid surface having a surface tension of 30-70 dyn/cm at 20°C.

In accordance with yet another embodiment, the surface tension is between 33-60 dyn/cm at 20°C and the eluted nucleic acid is RNA. In accordance to a further embodiment, the surface tension is between 38-60 dyn/cm at 20°C and the eluted nucleic acid is DNA.

In accordance with yet another embodiment, the organic solvent is an alcohol. In a preferred embodiment, the alcohol is ethanol, isopropanol, n-propanol, or butanol.

In yet another embodiment, the method comprising a further step of washing the solid support with a wash solution comprising an organic solvent miscible in water, prior

to eluting the nucleic acid. In accordance with a preferred embodiment, any step of the method is carried out at ambient temperature.

In accordance with yet another embodiment, the elution of the nucleic acid is in an aqueous liquid having a conductivity of up to 1 mS/cm. In accordance with a preferred embodiment, the elution liquid is water.

In accordance with the invention, a kit is provided for purification of a nucleic acid comprising

a container of washing solution comprising 30%-75% organic solvent miscible in water, and

a solid surface selected from the group consisting of an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20°C and an organic solid surface having a surface tension of 30-70 dyn/cm at 20°C,

wherein the kit components are used in a method for purification of nucleic acids wherein the nucleic acid is first precipitated onto the solid surface and then eluted from the solid surface at ambient temperature in an aqueous liquid having a conductivity of up to 1 mS/cm².

In accordance with an embodiment, the solid surface provided with the kit is an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20°C.

In accordance with another embodiment, the solid surface provided with the kit is an organic solid surface having a surface tension of 30-70 dyn/cm at 20°C.

In accordance with a preferred embodiment, the solid surface is borosilicate or cellulose acetate.

In accordance with a preferred embodiment, the surface tension of the solid surface is between 33-60 dyn/cm at 20°C and the eluted nucleic acid is RNA. In accordance with a preferred embodiment, the surface tension of the solid surface is between 38-60 dyn/cm at 20°C and the eluted nucleic acid is DNA.

DETAILED DESCRIPTION OF INVENTION

The invention is based on the unexpected observation that a nucleic acid in an aqueous sample solution can be purified by mixing the sample solution with a water miscible organic solvent in presence of a solid surface having a surface tension within a particular range and eluting the nucleic acid with good recovery in a primarily salt free liquid, wherein any step of the procedure can be carried at ambient temperature.

The method of purification, isolation and separation of nucleic acid from a solution sample comprises mixing an aqueous sample solution, organic solvent miscible in water, and an inorganic solid surface having surface tension of 38-70 dyn/cm in water at 20°C or an organic solid surface having surface tension of 30-70 dyn/cm at 20°C, collecting the solid surface on which the nucleic acid is precipitated, and eluting the nucleic acid.

The solid surface may, optionally, be washed prior to elution of the nucleic acid. The surface tension is a measure of the reversible work required to create a unit surface (interfacial) area at constant temperature, pressure, and composition. The surface tension is also referred to as an interfacial tension.

Although steps of the invention can be carried out at temperatures other than ambient temperature, any of the steps, including the elution step, can be conveniently carried out at ambient temperature. Ambient temperature is generally from about 18°C to about 28°C, and preferably from about 22°C to about 25°C.

The solid surfaces having surface tensions in the ranges described above are polar surfaces which interact with precipitated nucleic acid so that precipitated nucleic acid can stay on the solid surfaces, non specifically associated with the solid surface. Examples of

inorganic solid surfaces having surface tension of 38-70 dyn/cm in water at 20°C are borosilicates, aluminosilicates, aluminum oxides, and silicas. For the organic surfaces of the invention, the surface tension range is 30-70 dyn/cm at 20°C. Some examples of organic solid surfaces used in the invention are cellulose acetate, mixed cellulose esters, nylons, nitrocellulose, polyethylidene fluoride, polycarbonates, and polyethersulfone. The preferred range for organic solid surface is 38-60 dyn/cm at 20°C for purification of DNA. For purification of RNA, the preferred range is 30-60 dyn/cm. For example, cellulose acetate has a surface tension of 45.9 dyn/cm, nylon has a surface tension of 46.5 dyn/cm, nitrocellulose has a surface tension of 38 dyn/cm, and glass has a surface tension of 46 dyn/cm. The solid surfaces can be of a material having the desired surface tension, or chemically or physically modified solids to achieve a surface tension in the desired range. A skilled artisan knows how to determine the surface tension of a solid.

The solid surfaces may be in the forms of beads, particles, membranes, or filters. The order of mixing the sample solution, the water miscible organic solvent and the solid support is, generally, not important. For solid surfaces which are in the form of particles and beads, it is necessary that the solid support be well mixed with the sample and organic solvent liquids.

The sample solution may, in general, be used as it is at the end of some previous biological or chemical manipulation, without requiring any pre-treatment. The previous biological manipulation may be, for example, an extraction from a biological tissue or an enzymatic reaction. The sample solution may or may not contain salt. The sample solution may contain a mixture of nucleic acids, nucleotides, enzymes and proteins in general, carbohydrates, lipids, and generally, any component of a prior purification or

molecular manipulation of a biological sample. For example, the sample may be a cell lysate or a sequencing reaction. A sequencing reaction buffer may contain 80 mM Tris and 2 mM MgCl_2 . Any treatment that removes undesirable molecules from the sample solution prior to mixing the sample solution with the organic solvent miscible in water and solid support will not interfere with the purification method of the invention.

The organic solvents useful for the invention are water miscible, *i.e.* they form one phase when mixed in water. They may be alcohol, amide, ketone, acetonitrile, acetic acid, dimethyl formamide, dioxane, polyethylene glycol and the like. Preferred solvents are alcohol and acetonitrile. Preferred alcohols are ethanol, isopropanol, n-propanol, and butanol.

Starting concentration of the water miscible organic solvent may range 50–100%. A single such solvent or a mixture of the above solvents may be used in the invention. When water miscible organic solvent is mixed with the sample solution, one phase is formed. Sufficient organic solvent is added so the nucleic acid is separated from the solution and precipitated on the solid surface. The solid surface interacts with the precipitated nucleic acid so that the nucleic acid stays on the solid surface. The process can be conducted at ambient temperature.

In general, water miscible organic solvent is added to the aqueous sample solution so that 30–80 % final concentration of the organic solvent is achieved. Preferably, a 50–75% final concentration is achieved. In general, water miscible organic solvent can be added at ambient temperature. It may be added also at a lower temperature and it may be cooled to below ambient temperature prior to addition.

In accordance with the method of the invention, a sample solution is mixed with water miscible organic solvent and solid surfaces. The mixing may be by any mechanical action such as pipetting, inverting, or vortexing, etc. The solid surface with precipitated nucleic acid may be separated by decanting, filtration, centrifugation, vacuuming and the like. The optional wash step is carried out by addition of a water wash solution to the solid surfaces and then removal of the wash solution by decanting, filtration, centrifugation, vacuuming and the like.

The elution step comprises the addition of primarily salt free liquid and collection of the liquid. Water or a solution with a low ionic strength may be used. The elution step is preferably carried out at ambient temperature, in an elution solution which is at ambient temperature.

Wash step(s) may be included as desired. One or more wash steps may be carried out using the same or different wash solutions. Wash solution comprises a water miscible organic solvent diluted in water. Typically, the wash solution contains 0 – 70% water and 30 – 100 % water miscible organic solvent. The organic solvent may be the same as the miscible organic solvent initially mixed with the nucleic acid containing aqueous sample solution or it may be a different organic solvent. More than one water miscible organic solvents may be used as desired, in any one washing step or in subsequent washing steps. The wash solution is placed in contact with precipitated nucleic acid and removed by mechanical manipulation such as decantation, centrifugation, filtration, vacuuming, application of positive pressure, and the like.

To achieve the desired wash effect, the wash solutions may further comprise chelating agents, salts, acids or buffers. Examples of chelating agents, but not limited to

those examples, are EDTA, DCTA, and citrate. One or more than chelating agents may be used. The chelating agent may be used alone and in combination with other chelating agents. Examples of salt which can be included in the wash solution, but not limited to those examples are sodium chloride, or potassium acetate. One or more than one salt may be used. It can be used alone or with combination of others. An example of an acid which can be included in the wash solution, but not limited to this example is acetic acid. Examples of buffers, but not limited to those examples are Tris, Tris-acetate, sodium phosphate buffer, sodium citrate, sodium acetate and the like. One or more than one buffer may be used. It may be used alone or with combination of others. Examples of wash solutions are solutions containing 0.1 M NaCl alone; 10 mM Tris alone; 1 mM EDTA alone, or a combination thereof, such as, for example, 1mM Tris and 1 mM EDTA; 1 mM Tris and 1 mM $MgCl_2$; 1 mM EDTA and 10 mM KCl; 10mM sodium phosphate and 0.2 mM EDTA and 10 mM KCl. One or more than one wash steps may be performed. More than one wash solutions used may be same or may be different in each wash step. The wash steps can be carried out at ambient temperature.

The eluting solution is primarily salt free liquid. Preferred eluting solution is water. A low ionic strength may be used if desired. Low ionic strength solution may be, in general, any liquid that has a conductivity less than 1mS/cm. The ionic strength of a solution is measured as conductivity of the solution. The conductivity can be conveniently measured by use of a Digital Conductivity Meter (Fisher Scientific). An artisan skilled in the art knows how to measure conductivity of solutions. Eluting solutions are preferably used at ambient temperature. An example of a low conductivity

elution solution in accordance with the invention, but not limited to this example, would be 10 mM Tris and 1 mM EDTA which has about a 0.7 mS conductivity.

In a preferred embodiment, the solid surface is a filter or a membrane. These filters and membranes are porous solid which have average pore size range 0.1 – 5 μm . Preferred pore sizes range is 0.22 – 2 μm . In this embodiment, the sample solution containing nucleic acid is preferably mixed with water miscible organic solution first, and then the mixture is added to the filter or membrane. The sample solution and organic solvent solution need to be mixed well, not simply adding them together. The nucleic acids stay on the filter through non-specific interaction. The mixed nucleic acid and organic solution is passed through the filter or membrane by centrifugation, vacuuming, applying positive pressure (for example, syringe filtration) or gravity. A wash step may be included. The wash step comprises addition of a wash solution through the filter.

The eluting step generally includes removal of nucleic acid from the filter. The elution liquid is water or a low salt concentration buffer. It is added to the filter and passed through the filter or membrane into a clean collection vessel, or the membrane or filter are rinsed in the elution liquid and the elution liquid which contains the nucleic acid is collected. This whole process is preferably carried out at ambient temperature.

The nucleic acid in the sample which is purified in accordance to the invention is any molecule made of either deoxyribonucleotide or ribonucleotide monomers. Increased organic solvent concentrations will allow recovery of even very short (2 mer) oligonucleotides. Examples of nucleic acids which can be purified by the invention include DNA, DNA fragments, double strands DNA, single strand DNA, RNA, DNA or RNA fragments from amplification reactions and restrictions, oligonucleotides, modified

RNA or DNA, or any of the above molecules comprising a modified or labeled nucleotide, or having a label attached thereto, such as a biotin tagged oligonucleotide, fluorescence-tagged DNA, a oligonucleotide or ribonucleotide conjugated with other molecules.

The sample solution is, or can be derived from, any artificial or biological source. For example, the sample can be from a chemical reaction creating synthetic oligonucleotides, a cell culture, a biological tissue, blood, serum, a fermentation broth, plasma, milk, or a body fluid. The sample can be used either directly or following a treatment before use. Examples of pretreatments include disrupting cells and removing cell debris, preparing plasma from, preparing liquid from solid materials, concentrating and diluting the fluids, modifying through chemical or enzyme reactions and the like. An artisan skilled in the art will understand that a sample solution can, therefore, contain a limited amount of organic solvents, typically no more than 10%. Such a solution continues to be the aqueous solution from which nucleic acids are to be purified, in accordance with the invention. The purification of the nucleic acid can be followed by methods well known in the art such as, for example, a UV spectrometer, gel electrophoresis, or radiation detection if the nucleic acid is labeled with isotopes.

The following are examples illustrating the present invention and are intended to be illustrative and not intended to limit the invention.

Examples

Example 1 *Purification of fluorescent oligonucleotide*

To 30 μ l PCR buffer containing 0.5 μ g 16-mer fluorescent oligonucleotide, 70 μ l ethanol were added and mixed. Seven equivalent samples were prepared and transferred, one sample each of :

1. glass fiber filter membrane
2. cellulose acetate filter membrane
3. nylon filter membrane
4. aluminum oxide filter membrane
5. polypropylene filter membrane
6. polyvinylidene fluoride filter membrane
7. nitrocellulose filter membrane

A brief centrifugation to remove the liquid followed. We observed that supernatants from filters 1, 2, 3 and 4 have no fluorescence and supernatants from filters 5, 6 and 7 are fluorescent and their strengths are close to original solution. The bound fluorescent oligonucleotide was seen with naked eyes on filters 1, 2, 3 and 4. The oligonucleotides were eluted with 20 μ l water run on 1.2 % agarose gel and visualized by staining with ethidium bromide. Unpurified 16-mer oligonucleotide was used as a control. The gel image showed that filters 1, 2, 3 and 4 gave almost 100% yield. However, there are almost no oligonucleotide recovered from filters 5 and 6 and a very low recovery rate was obtained from nitrocellulose membrane.

Example 2 Purification of MspI digested pBR322 DNA

40 μ l ethanol was added to 20 μ l restriction buffer containing 0.5 μ g MspI digested pBR322 DNA. Six such samples were prepared and transferred to different filter membranes:

1. glass fiber filter membrane
2. cellulose acetate filter membrane
3. nylon filter membrane
4. aluminum oxide filter membrane
5. polypropylene filter membrane
6. polyvinylidene fluoride filter membrane.

The filters were centrifuged for one minute at 700 xg and washed once with 70% ethanol. The DNA was eluted with 20 μ l water. The samples were run on 1.0 % agarose gel and the DNA was visualized by staining with ethidium bromide. Unpurified MspI digested pBR322 DNA was used as a control. The gel image shows all the DNA bands were recovered with almost 100% yield on filters 1, 2, 3 and 4. However, almost no DNA was recovered from filters 5 and 6.

Example 3 Purification of λ -DNA

30 μ l ethanol was added to 20 μ l restriction buffer containing 0.5 μ g λ -DNA. Two such samples were prepared, mixed and transferred to different filter membranes:

1. glass fiber filter membrane
2. cellulose acetate filter membrane

The filters were centrifuged one minute at 700 xg, washed once with a 70% ethanol containing 1 mM EDTA solution and once with a 70% ethanol solution. Eluted with 20 μ l water. The samples were run on 1.0 % agarose gel and the DNA was visualized by staining with ethidium bromide. Unpurified λ -DNA was used as a control. The gel image shows that filters 1 and 2 give almost 100% yield.

Example 4 *Purification of DNA from a sequencing reaction*

Standard sequencing reaction were performed using a PE Biosystems: The ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit. PGEM-3Zf (+) double stranded template and -21 M13 primer (forward) were used for the sequencing reaction. 10 μ l of water and 45 μ l of isopropanol or ethanol were added to the reaction solution and mixed. The mix was transferred to glass fiber filters and to cellulose acetate membranes. Vacuum was applied to remove supernatants. The solid support was washed twice with 55% isopropanol. The DNA was eluted with 20 μ l of water. The samples were then sequenced on an ABI 3700 sequencer. The graphs showed strong DNA signals and removal of terminator dye.

Example 5 *Another protocol for purification of DNA from a sequencing reaction*

Standard sequencing reactions were performed using a PE Biosystems: The ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit. PGEM-3Zf (+) double stranded template and -21 M13 primer (forward) were used for the sequencing reaction. 10 μ l of water and 50 μ l of 95% ethanol were added to the reaction solution and mixed. The mix was transferred to glass fiber filters and to cellulose acetate membranes.

Vacuum was applied to remove supernatants. The solid support was washed once with 70% ethanol containing 0.5mM EDTA and once with 70% ethanol. The DNA was eluted with 20µl of water. The samples were then sequenced on an ABI 3700 sequencer. The graphs showed strong DNA signals and removal of dye terminator.

Example 6. *Purification of plasmid DNA from a cell culture*

2 ml DH5α (host *E. Coli*) with PUC19 vector (from Life Technologies Inc.) were cultured overnight in LB (Lucia Broth) and centrifuged at 14,000 xg for 30 seconds to remove supernatant. The cells were resuspended in 60 µl of 1 x TE (Tris-EDTA buffer). 30 µl of lysis buffer containing 0.25 M NaOH and 1% SDS was added and mixed in by gently inverting until the cells were completely lysed (5-10 minutes). 20 µl of 3M potassium acetate (pH 4) was then added. The resulting mix was centrifuged at 14,000 x g to precipitate the pellet. 150 µl isopropanol was added and mixed well. The solution was transferred to a filter cartridge with glass fiber and centrifuged to remove the supernatant. The solid support was washed twice with a solution containing 50% isopropanol, 10 mM Tris, and 1mMEDTA. The DNA was eluted with 50 µl water. 10 µl samples were run on a 1% agarose gel, which indicated a good recovery rate.

I claim:

1. A method of purification of a nucleic acid from a solution comprising:
mixing an aqueous sample solution comprising a nucleic acid, an organic solvent miscible in water, and a solid surface selected from the group consisting of an inorganic solid surface having a surface tension of 38-70 dyn/cm measured in water at 20⁰C and an organic solid surface having a surface tension of 30-70 dyn/cm measured at 20⁰C;
collecting the solid surface on which the nucleic acid is precipitated; and
eluting the nucleic acid from the solid surface at ambient temperature.
2. The method of claim 1, wherein the solid surface is an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20⁰C.
3. The method of claim 1, wherein the solid surface is an organic solid surface having a surface tension of 30-70 dyn/cm at 20⁰C.
4. The method of claim 1, wherein the solid surface is borosilicate, cellulose acetate, nylon, or aluminum oxide.
5. The method of claim 4, wherein the solid surface is borosilicate or cellulose acetate.
6. The method of claim 3, wherein the surface tension is between 33-60 dyn/cm measured at 20⁰C and the eluted nucleic acid is RNA.
7. The method of claim 3, wherein the surface tension is between 38-60 dyn/cm measured at 20⁰C and the eluted nucleic acid is DNA.
8. The method of claim 1, wherein the organic solvent is an alcohol.
9. The method of claim 8, wherein the alcohol is ethanol, isopropanol, n-propanol, or butanol.
10. The method of claim 1 comprising a further step of washing the solid support with a wash solution comprising an organic solvent miscible in water, prior to eluting the nucleic acid.
11. The method of claim 1 or 10, wherein any step of the method is carried out at ambient temperature.

12. The method of claim 1, wherein said nucleic acid is single stranded, double stranded, RNA, DNA, RNA-DNA hybrids, a modified nucleic acid, a tagged nucleic acid, or an oligonucleotide.

13. The method of claim 1, wherein the elution is in an aqueous liquid having a conductivity of up to 1mS/cm.

14. The method of claim 13, wherein said aqueous liquid is water.

15. A kit for purification of a nucleic acid comprising:

a container of washing solution comprising 30%-70% organic solvent miscible in water, and

a solid surface selected from the group consisting of an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20⁰C and an organic solid surface having a surface tension of 30-70 dyn/cm at 20⁰C,

wherein the kit components are used in a method for purification of nucleic acids wherein the nucleic acid is first precipitated onto the solid surface and then eluted from the solid surface at ambient temperature in an aqueous liquid having a conductivity of up to 1 mS/cm².

16. A kit in accordance with claim 15, wherein said solid surface is an inorganic solid surface having a surface tension of 38-70 dyn/cm in water at 20⁰C.

17. The kit of claim 15, wherein said solid surface is an organic solid surface having a surface tension of 30-70 dyn/cm at 20⁰C.

18. The kit of claim 15, wherein the solid surface is borosilicate or cellulose acetate.

19. The kit of claim 17, wherein the surface tension is between 33-60 dyn/cm at 20⁰C and the eluted nucleic acid is RNA.

20. The kit of claim 17, wherein the surface tension is between 38-60 dyn/cm at 20⁰C and the eluted nucleic acid is DNA.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST AND STN BIOTECH FILES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 6,084,091 A (MULLER et al) 04 July 2000, Col. 4 lines 49-65	1-20N
Y	US 5,990,301 A (COLPAN et al) 23 November 1999, entire document.	1-20
Y	US 6,037,465 A (HILLEBRAND et al) 14 March 2000, entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* - Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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A. CLASSIFICATION OF SUBJECT MATTER:

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